REMARKS

In the previously filed Reply to Final Office Action (filed on November 13, 2007), applicants requested that claims 22, 23, and 24 be amended. The Advisory Action mailed November 28, 2007 indicated that these amendments would not be entered. Applicants therefore request that the claim amendments presented here be entered and that the November 13, 2007 amendments not be entered.

Upon entry of the proposed amendments, claims 22, 24, and 33-45 will be pending and under examination. Claims 1-21, 23, and 25-32 have been canceled. Applicants have amended claim 22 to recite a method of treating undesirable muscle contraction that results from exaggerated release of acetylcholine from pre-synaptic nerve terminals and to specify that the composition is administered in an amount sufficient to reduce acetylcholine release from presynaptic nerve terminals in the patient. Applicants have amended claim 24 to make it an independent claim and added new claims 38-45. Support for the amendments can be found throughout the specification and in the original claims. Support for the amendment to claim 22 can be found, for example, in the specification page 12, lines 12 and in cancelled claim 23. Support for the amendment to claim 24 can be found in the original claim. Support for new claim 38 can be found, for example, in the specification at page 12, lines 12-16. Support for new claim 39 can be found in original claim 25. Support for new claim 40 can be found in original claim 26. Support for new claim 41-45 can be found in previously presented claims 34-37. No new matter has been added.

35 U.S.C. §112, Indefiniteness

The Final Office Action mailed June 12, 2007 (the "Office Action") maintained a prior rejection of claims 22-24 as allegedly indefinite and rejected newly entered claims 33-37. Examiner Chih Min Kam conducted an interview with applicants' representatives on December 21, 2007 to discuss this rejection.

Based on the interview, applicants' representatives understood that Examiner Kam's primary concern was that a disease or condition "associated" with exaggerated release of acetylcholine might not necessarily result from the exaggerated release of acetylcholine. That is,

Examiner Kam seemed to be concerned about diseases or conditions wherein the symptoms are not caused by exaggerated acetylcholine release but are nevertheless "associated" with it. While applicants do not agree, in an attempt to address the Examiner's concerns, applicants have amended claim 22 to recite a method of treating "undesirable muscle contraction that results from exaggerated release of acetylcholine from presynaptic nerve terminals."

Applicants submit that a person of ordinary skill would have understood the metes and bounds of amended claim 22 at the time of this application's filing. At the time the present application was filed, it was known that acetylcholine binding to a muscle cell at a neuromuscular junction causes that muscle cell to contract. This is evident in a college-level textbook (Bruce Alberts et al., Molecular Biology of the Cell, 3rd edition, p. 540, 1994, Exhibit A), which describes the mechanism by which acetylcholine induces muscle contraction. A person of ordinary skill would have understood that acetylcholine released from presynaptic nerve terminals (at neuromuscular junctions) binds to receptors on muscle cells inducing changes in cytosolic Ca^{2+} that cause myofibrils in the muscle cells to contract. A person of ordinary skill would have understood that "undesirable muscle contraction that results from exaggerated release of acetylcholine from presynaptic nerve terminals," refers to muscle contraction that occurs via this mechanism.

Furthermore, a person of ordinary skill would have been aware that Botulinum neurotoxin inhibits acetylcholine release from pre-synaptic nerve termini and that it could inhibit undesirable muscle contraction. (For example, see Lew et al. ("Review of the FDA-approved uses of botulinum toxins, including data suggesting efficacy in pain reduction", Clin. J. Pain., 18:S142-6, 2002 (Exhibit B at page S142, abstract) and Poungvarin et al. "Treatment of Various Movement Disorders with Botulinum A Toxin Injection: An Experience of 900 Patients", J. Med. Assoc. Thai., 78:281-288, 1995 (Exhibit C at page 281, col. 2, para. 1, to page 281, col. 1, para. 2)).

Given the state of the art (as reflected by the clinical use of Botulinum neurotoxin to inhibit undesirable muscle contraction), and the teachings of the specification (for example, at page 12, lines 11-12) at the time of filing, a skilled practitioner would have clearly understood the metes and bounds of amended claim 22. Applicants respectfully request that the present rejection be withdrawn.

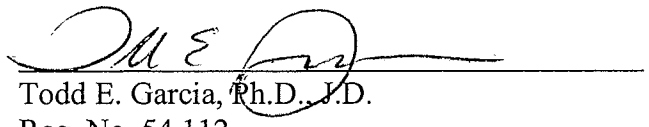
With regard to claim 24, which recites methods of treating "sweating, lacrimation, or mucous secretion that result from exaggerated release of acetylcholine from presynaptic nerve terminals," applicants submit that it was known in the art that acetylcholine binding to receptors regulates the production of biological fluids. For example, acetylcholine was known to regulate eccrine gland cells, which produce sweat. Kreyden and Scheidegger ("Anatomy of the sweat glands, pharmacology of botulinum toxin, and distinctive syndromes associated with hyperhidrosis", Clin. Dermatol., 22:40-4, 2004, attached hereto as Exhibit D) describe how acetylcholine binding to receptors on eccrine gland clear cells regulates sweating. Furthermore, Kreyden and Scheidegger discuss treating sweating using Botulinum neurotoxin (at page 40, col. 1, para. 2), demonstrating that a skilled practitioner would have understood that Botulinum neurotoxin could be used to regulate the production of biological fluids.

For at least these reasons, applicants submit that the metes and bounds of the present claims are completely clear. Applicants therefore request that the rejection be reconsidered and withdrawn.

The amount of $930 for the Petition for Extension of Time fee and the Request for Continued Examination fee is being submitted via the Electronic Filing System by Deposit Account Authorization. Please apply any other charges or credits to deposit account 06-1050, referencing Attorney Docket No. 08387-002003.

Respectfully submitted,

Date: 9/14/08



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Neuromuscular Transmission Involves the Sequential Activation of Five Different Sets of Ion Channels [15]

The importance of ion channels to electrically excitable cells can be illustrated by following the process whereby a nerve impulse stimulates a muscle cell to contract. This apparently simple response requires the sequential activation of five different sets of ion channels—all within a few milliseconds (Figure 11–34).

1. The process is initiated when the nerve impulse reaches the nerve terminal and depolarizes the plasma membrane of the terminal. The depolarization transiently opens voltage-gated Ca^{2+} channels in this membrane. As the Ca^{2+} concentration outside cells is more than 1000 times greater than the free Ca^{2+} concentration inside, Ca^{2+} flows into the nerve terminal. The increase in Ca^{2+} concentration in the cytosol of the nerve terminal triggers the localized release of acetylcholine into the synaptic cleft.
2. The released acetylcholine binds to acetylcholine receptors in the muscle cell plasma membrane, transiently opening the cation channels associated with them. The resulting influx of Na^+ causes a localized membrane depolarization.
3. The local depolarization of the muscle cell plasma membrane opens voltage-gated Na^+ channels in this membrane, allowing more Na^+ to enter, which further depolarizes the membrane. This, in turn, opens neighboring voltage-gated Na^+ channels and results in a self-propagating depolarization (an action potential) that spreads to involve the entire plasma membrane (see Figure 11–23).
4. The generalized depolarization of the muscle cell plasma membrane activates voltage-gated Ca^{2+} channels in specialized regions (the transverse [T] tubules—discussed in Chapter 16) of this membrane. This, in turn, causes *Ca^{2+} release channels* in an adjacent region of the sarcoplasmic reticulum membrane to open transiently and release the Ca^{2+} stored in the sarcoplasmic reticulum into the cytosol. It is the sudden increase in the cytosolic Ca^{2+} concentration that causes the myofibrils in the muscle cell to contract. It is not certain how the activation of the voltage-gated Ca^{2+} channels in the T-tubule membrane leads to the opening of the Ca^{2+} release channels in the sarcoplasmic reticulum membrane. The two membranes are closely apposed, however, with the two types of channels joined together in a specialized structure (see Figure 16–92). It is possible, therefore, that a voltage-induced change in the conformation of the plasma membrane Ca^{2+} channel directly opens the Ca^{2+} release channel in the sarcoplasmic reticulum through a mechanical coupling (discussed in Chapter 16).

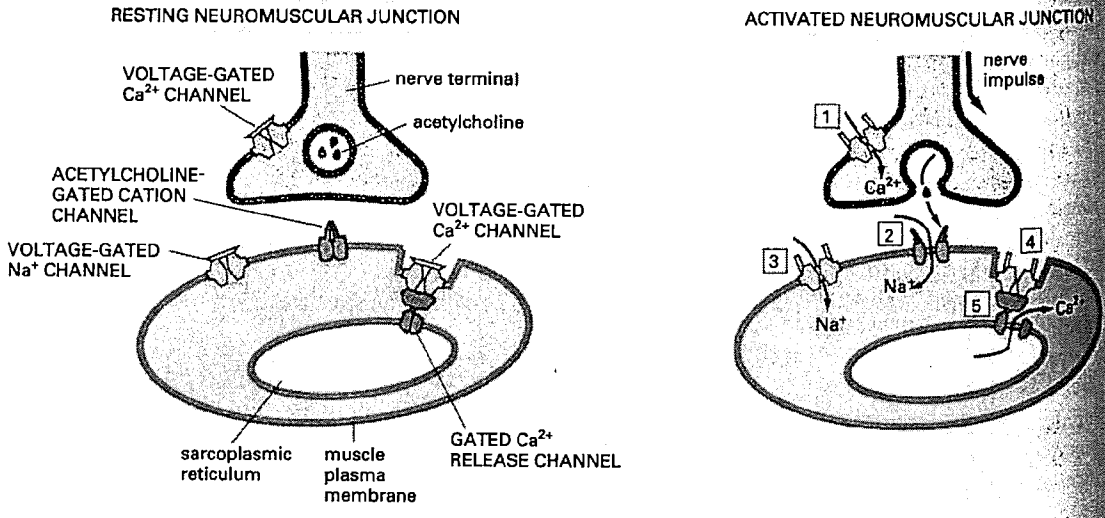

Figure 11–34 **The system of ion channels at a neuromuscular junction.** These gated ion channels are essential for the stimulation of muscle contraction by a nerve impulse. The various channels are numbered in the sequence in which they are activated, as described in the text.

EXHIBIT B

THE CLINICAL JOURNAL OF PAIN

BOTULINUM TOXIN FOR PAIN MANAGEMENT AND OTHER MEDICAL CONDITIONS: A COMPREHENSIVE REVIEW

GUEST EDITOR:
Charles E. Argoff, MD

VOLUME 18 • NUMBER 6
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Volume 18, Number 6, November/December 2002 Supplement

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Review of the FDA-Approved Uses of Botulinum Toxins, Including Data Suggesting Efficacy in Pain Reduction

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Abstract:

Botulinum toxin has dramatically improved the treatment of a variety of neurologic disorders. Two botulinum toxin preparations are commercially available in the United States: type A (Botox) and type B (Myobloc). Current indications approved by the United States Food and Drug Administration include cervical dystonia, strabismus, blepharospasm, hemifacial spasm, and glabellar wrinkles for Botox, and cervical dystonia for Myobloc. Botulinum toxin inhibits release of acetylcholine from the neuromuscular junction, resulting in a localized paralysis when minute doses are injected. This mechanism enables botulinum toxin to alleviate symptoms of focal dystonias (which are characterized by excessive muscle contraction), and it may also, along with other theoretical mechanisms, be responsible for pain relief. Studies conducted in patients with cervical dystonia have shown that botulinum toxin effectively reduces pain associated with this disorder, suggesting that this agent may be effective in alleviating other painful syndromes.

Key Words: Analgesia—Blepharospasm—Botulinum toxin—Botox—Cervical dystonia—Hemifacial spasm—Myobloc—Pain—Strabismus

Botulinum toxin injections are effective for many clinical disorders that involve involuntary muscle activity or increased muscle tone. Botulinum toxin is a presynaptic neuromuscular blocking agent. It produces a temporary chemical denervation via blockade of acetylcholine release from motor nerve terminals. Muscle weakness occurs within a few days to 1 week after local injection, peaks within 2 weeks for several weeks, and then plateaus in milder form (the desired clinical effect) before gradually returning to baseline. Recovery from toxin-induced paralysis involves resprouting of terminals from the axon, followed by slow recovery of the neuron's ability to release acetylcholine.[1,2] The clinical effects may last for 3 to 4 months after each injection. The dose administered affects both the intensity of denervation and the length of the initial and plateau periods.[1,3–6]

Dr. Alan Scott was the first to use botulinum toxin therapeutically in the 1970s, treating surgically produced strabismus in monkeys and then strabismus in humans.[7] After more than a decade of research, the United States Food and Drug Administration (FDA) approved the type A toxin (Botox; Allergan Pharmaceuticals, Irvine, CA) for the treatment of strabismus, blepharospasm, and seventh cranial nerve disorders (hemifacial spasm) in 1989. More than a decade later, in December of 2000, the immunologically distinct type B toxin (Myobloc; Elan Pharmaceuticals, San Diego, CA) was approved for cervical dystonia. Soon thereafter, Botox also received FDA approval for treatment of cervical dystonia and, most recently, glabellar wrinkles. Outside the United States, a different preparation of the type A toxin (Dysport; Ispen, Ltd., Berkshire, UK) is available. Myobloc is also available in European countries under the trade name NeuroBloc (Elan Pharmaceuticals, San Diego, CA).

This article reviews the use of botulinum toxin for the FDA-approved indications of strabismus, blepharospasm, hemifacial spasm, and cervical dystonia. Use of this agent in the treatment of glabellar wrinkles is

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discussed elsewhere in this supplement. In addition, this article provides additional data suggesting the effectiveness of botulinum toxin in providing pain relief.

STRABISMUS

Botulinum toxin was first used for medicinal purposes in the 1970s to produce weakness in the lateral rectus eye muscle of monkeys with experimentally induced strabismus. The idea was to restore the balance between antagonistic pairs of extraocular muscles. In these initial experiments, the toxin was injected directly into the extraocular muscle of the deviated eye, weakening the overactive muscle and resulting in realignment of the optical axis. These successful initial trials led to the use of botulinum toxin to treat strabismus in humans.[7,8] Strabismus is the most rigorously studied ophthalmologic indication for botulinum toxin. Studies show that botulinum toxin type A reduces ocular deviation in more than 50% of patients.[9–11] The recommended dosage ranges for botulinum toxin type A is 1.5 to 2.5 units in any single muscle and up to 5 units for larger deviations. Botulinum toxin may be used alone, as an acceptable alternative to avoid or delay surgery, or in conjunction with surgery.[3] Reported adverse events have been rare and are usually temporary. Complications include transient ptosis, subconjunctival hemorrhage, and transient vertical deviations of the globe.[5,12]

BLEPHAROSPASM

Blepharospasm is a focal dystonia that causes progressive involuntary spasms and forced closure of the eyelid muscles (orbicularis oculi).[13] Onset of the disorder is usually characterized by an increase in blinking or squinting, followed by forceful closure of and difficulty in opening the eyelids. In many cases, blepharospasm affects the patient's ability to read or drive. In severe cases the patient may become functionally blind. Spasms may extend into the midfacial and low facial muscles, and sometimes to the jaw and neck. Previous methods of treatment, including oral medications and surgical denervation or myectomy of the orbicularis oculi, have not been satisfactory. Physical means, such as crutches attached to custom-made spectacles, have helped only a small number of patients.[5] Doxorubicin injection remains experimental and is associated with intolerable side effects.

Botulinum toxin is the treatment of choice for blepharospasm. Clinical studies of the type A toxin show that it is effective in more than two-thirds of patients.[14] The usual dose of botulinum toxin type A is 12.5 to 25 units per eye; some patients require higher doses (30 to 75 units) to achieve spasm control. Preliminary reports also indicate efficacy with botulinum toxin type B.[15] Guidelines suggest doses of 750 to 2,500 units in one to three sites per side.[16] Treatment reduces muscle spasms and associated sensory complaints (e.g., photophobia, foreign body sensation in the eyes). As in other dystonic conditions, the effects of botulinum toxin last an average of 12 weeks before reinjection is necessary. Adverse effects are usually a result of the chemodenervating properties of botulinum toxin on adjacent muscle groups and include ptosis, diplopia, midfacial weakness, and epiphoria.[1–3,12]

HEMIFACIAL SPASM

Caused by vascular irritation or compression of the facial nerve leading to the brainstem, hemifacial spasm is characterized by intermittent clonic or tonic contractions of the muscles supplied by the facial nerve. It is usually a chronic, progressive disease, and generally presents unilaterally.[17] One study showed that, among 158 patients with hemifacial spasm, 41% reported social embarrassment, 39% reported interference with vision, and 11% reported some discomfort or pain.[18] In this study, carbamazepine was the most commonly prescribed medication, followed by clonazepam, baclofen, other benzodiazepines, anticonvulsants, and anticholinergics. Only 8% of study participants reported meaningful benefit from any of these agents. Surgical microvascular decompression of the facial nerve can effectively cure the condition in most cases, but serious potential complications, such as permanent facial weakness or hearing loss, deter many patients from undergoing this procedure.[2]

Botulinum toxin has become the treatment of choice for hemifacial spasm.[2,5] Almost all patients show substantial improvement, and the effects appear to last for up to 5 months. The most common adverse effects are transient ptosis and facial weakness.[12,17,18] The initial dose of Botox is 1.25 to 2.5 units (0.05 to 0.1 ml) injected into abnormally contracting muscles, rarely exceeding 5 units in a single location. For botulinum toxin type B, the initial dose is 125 to 250 units per muscle site (total dose 750 to 5,000 units).[16]

CERVICAL DYSTONIA

The most common form of focal dystonia, cervical dystonia, affects 60,000 to 90,000 persons in the United States.[19] This disorder is characterized by abnormal, involuntary contractions of the cervical and/or shoulder muscles, resulting in twisting movements and abnormal postures of the head. Unlike other focal dystonias, cervical dystonia is associated with significant musculoskeletal pain as a result of the repetitive twisting and turning

movements.[20] Symptoms of cervical dystonia compromise the patient's ability to lead a normal life and have an adverse impact on psychological wellbeing.[21]

Cervical dystonia does not respond in a robust fashion to conventional pharmacologic or surgical treatments. For many years, oral pharmacologic agents, including anticholinergics, dopamine-depleting and dopamine-blocking agents, and other muscle relaxants, were used as first-line therapy. However, most patients have troublesome side effects from therapeutic doses of virtually all these medications, and the benefits tend to decrease over time.[22,23] Nonpharmacologic modalities (e.g., physical therapy, hypnosis, biofeedback, acupuncture) have also been used, but with limited success.[24] Surgical denervation has limited benefit and is usually reserved for patients in whom other forms of treatment have failed. Ten percent to 20% of patients go into remission but almost all relapse.[5,25]

Botulinum toxin injections, which are considered primary therapy for patients with cervical dystonia, improve head positioning, pain, and disability in up to 90% of patients.[12,25] A large body of data supporting the efficacy of the type A toxin has been published, although most studies have consisted of small cohorts or were not well controlled.[26] Patients treated for up to 10 years have continued to respond to therapy.[4] Adverse effects include dysphagia, neck weakness, and cervical and shoulder/hand pain. The dose of botulinum toxin type A required to effectively treat each muscle has varied according to the study performed. In typical cases, the total dose ranged from 100 to 300 units. Of importance is that dosing should be individualized and adjusted according to certain dose-modifying factors, as listed in Table 1. It is generally agreed that doses greater than 400 units should be avoided.[27] High doses, as well as more frequent reinjections (at intervals of less than 3 months) and longer treatment duration, have been associated with the development of antibodies that neutralize the toxin's effects, conferring immunoresistance. According to retrospective and anecdotal reports, 3% to 10% of patients treated with botulinum toxin type A develop resistance.[28]

TABLE 1. *Dose-modifying factors for botulinum toxin*

Clinical situation	Start at the low end of the range if	Start at the high end of the range if
Patient weight	Low	High
Muscle bulk	Very small	Very large
Disease severity	Mild	Severe
Concern for weakness	High	Low
Results of previous therapy	Too much weakness	—

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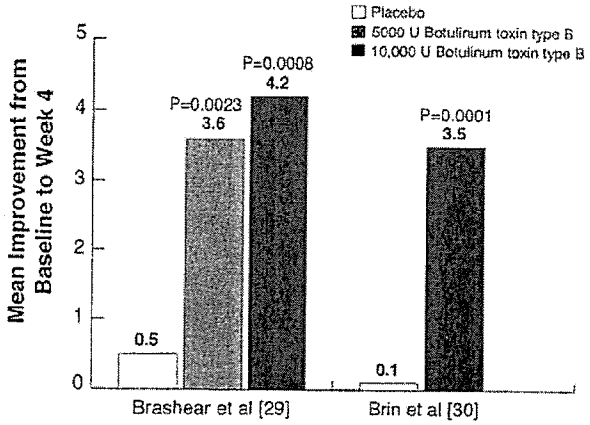

FIG. 1. Results of TWSTRS-Pain subscale scores (mean improvement from baseline week 4) in two randomized, placebo-controlld trials of botulinum toxin type B.

Perhaps the most convincing data supporting the effectiveness of botulinum toxin in reducing pain are derived from clinical trials that evaluated the type B toxin for cervical dystonia. Two randomized, double-blind, placebo-controlled clinical trials have confirmed its efficacy in cervical dystonia.[29,30] The benefit was found to extend even to patients with confirmed resistance to botulinum toxin type A.[30] In these trials, the most common adverse effects of botulinum toxin type B were dysphagia and dry mouth, which were mild to moderate in severity and were self-limited. The two trials specifically evaluated pain outcomes by obtaining Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) pain subscale scores as well as Patient Analog Pain scores. In both pain measurements, botulinum toxin type B was significantly more effective than placebo (Figs. 1 and 2). Effective doses for botulinum toxin type B range from 5,000 to 10,000 units and, like type A, should be modified as shown in Table 1. At therapeutic doses, the clinical benefits of botulinum toxin type B last for 12 to 16 weeks, which is similar to the duration reported with the type A toxin.[31] The prevalence of immunoresistance in patients who have received botulinum toxin type B is unknown. However, a large prospective, long-term, open-label trial conducted in more than 400 patients with cervical dystonia has recently been completed and may add important knowledge about resistance to botulinum toxin type B and to botulinum toxins in general.

OTHER PAIN SYNDROMES

The effectiveness of botulinum toxin in relieving pain from cervical dystonia has led to the exploration of its use in other pain syndromes. Conditions now under study include chronic tension-type headache, migraine, myofascial pain, and painful spasticity. In these disorders,

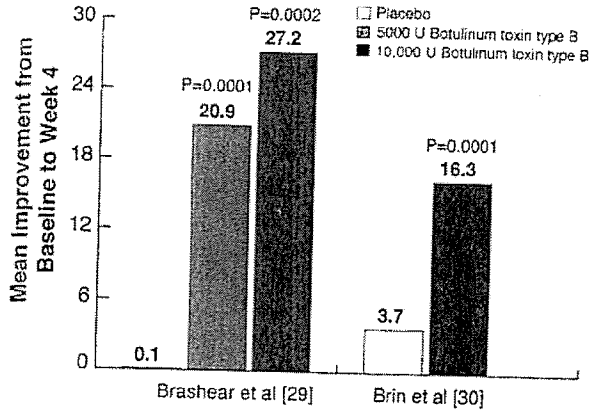

FIG. 2. Results of Patient Analog Pain (mean improvement from baseline to week 4) in two randomized, placebo-controlled trials of botulinum toxin type B.

direct involvement of hyperactive muscles is difficult to demonstrate, suggesting that botulinum toxin may provide pain relief through a mechanism other than chemodenervation.[32] Preliminary results of botulinum toxin in these disorders are promising.[33–35]

SUMMARY

Botulinum toxin is a safe and effective treatment for strabismus, blepharospasm, hemifacial spasm, and cervical dystonia. It also has demonstrated benefit in many other neurologic and nonneurologic disorders. The benefit of botulinum toxin for its approved indications is clearly related to its ability to cause muscle paralysis in overactive dystonic muscles. Dosage should be selected on an individualized basis, depending on the disorder being treated, the size of the muscle, and various other factors. Guidelines for appropriate administration of botulinum toxin are provided in Table 2.

Results from studies have also demonstrated that botulinum toxin provides significant pain relief in patients with cervical dystonia.[29,30] It has been reported that the pain relief attained in these patients exceeds the measurable effect on the injected muscle and sometimes occurs even before any change in muscular pattern is observed.[36] These results suggest that mechanisms other than chemodenervation may be involved. For example, noncholinergic neurotransmitter involvement, including modulation of substance P, has been postulated to account for the pain relief.[37] As a result of these studies, botulinum toxin is now being investigated in difficult-to-treat painful conditions such as migraine and myofascial pain. The preliminary results are thus far very promising.

TABLE 2. *Guidelines for administration of botulinum toxin*

Consider if botulinum toxin therapy is appropriate for the patient and condition being treated
Determine which muscles need to be injected
Determine the appropriate dosage, and the number and volume of injections per session
Use the smallest effective total dose and volume
Use appropriate techniques to achieve precise injection and reduce the risk for complications
Follow up with patient to assess efficacy, safety, and satisfaction with treatment
Record details of treatment (dose, volume, sites) and patient response to treatment to guide future injections
Administer subsequent injection with as long an interdose interval as possible
Reassess the treatment regimen and the patient's response (both positive and negative)

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EXHIBIT C

THAILAND

วารสารแพทย์ แพทยสมาคมแห่งประเทศไทย

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Treatment of Various Movement Disorders with Botulinum A Toxin Injection : An Experience of 900 Patients

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Botulinum toxin type A, currently used mostly for therapeutic purposes, is harvested from a culture medium after fermentation of a high toxin-producing strain of *Clostridium botulinum*, which lyses and liberates the toxin into the culture(1). The toxin is then extracted, precipitated, purified and finally crystallized with ammonium sulfate. The crystalline toxin is diluted from milligram to nanogram concentrations, freeze-dried, and dispensed as a white power in small vials. There are seven immunologically distinct toxins A, B, C, D, E, F. The type A has been studied most intensely and is used most widely, but the clinical applications of other types of toxins including B and F are also being explored(2-4).

The therapeutic value of botulinum toxin (BTX) is due to its action at the neuromuscular junction to cause chemodenervation and to produce local paralysis when injected into a muscle (5,6). Recent studies have elucidated the intracellular mechanisms of action of the various BTX serotypes. Schiavo et al demonstrated that the heavy chain BTX-B binds to the presynaptic cholinergic terminal and the light chain, zinc endopeptidase, prevents the quental release of acetylcholine by proteolytically cleaving vesicle associated membrane protein (VAMP) (also known as synaptobrevin); and integral protein of the synaptic vesicle membrane(6). BTX-D and BTX-F also appear to cleave VAMP but at different sites(7). In contrast, BTX-A and BTX-E have been found to inhibit acetylcholine release from the presynaptic neuromuscular terminal by cleaving synaptosomal associated protein (SNAP-25); BTX-C acts by cleaving syntaxin(8). The chemodenervation causes the muscle to atrophy. The process is reversed within 2 to 4 months as the nerve sprouts and reinnervates the muscle. Multiple injections into the target muscle appear to be more efficacious and are associated with a lower incidence of complications than a single injection(9,10).

Clinical application of BTX-A was first used as an alternative to surgery for strabismus (11). Later, BTX-A was proven by double-blind, placebo controlled studies as an effective treatment for blepharospasm, spasmodic torticollis, and hemifacial spasm(12-15). BTX-A injection is now accepted as the standard treatment of choice for ormandibular-facial-lingual dystonia, task-specific dystonia (occupational cramps), and spasmodic dysphonia(16-20). Recently the usefulness of BTX-A was documented for various movement disorders such as tremor (voice, head and limb), palatal myoclonus, and tics(21-23). Inappropriate contraction of muscles such as nystagmus, myokimia, bruxism, stuttering, painful rigidity, muscle

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contraction headaches, lumbosacral strain with back muscle spasms, radiculopathy with secondary muscle spasm, foot dystonia, spasticity, post-traumatic muscle spasms, stiff-person syndrome, spastic bladder, achalasia, and anismus also benefited from BTX-A injection[22,24-27]. Other potential applications for BTX-A were protective ptosis, cosmetic for wrinkles and facial asymmetry[22].

Possible contraindications for the use of BTX include the presence of myasthenia gravis, Eaton-Lambert syndrome, motor neurone disease, aminoglycoside antibiotic use, and pregnancy. Besides occasional complications, usually related to local weakness, the major limitation of BTX therapy is its high cost. In Thailand, Siriraj Hospital has been the only collaborative centre of BTX in South East Asia to Smith-Kettlewell Eye Research Institute, San Francisco since 1989 (before the American Food and Drug Adminstration approval of BTX). We thus have six years of uninterrupted experience of BTX-A treatment for various movement disorders in Thailand and our data are worthwhile for analyses.

MATERIAL AND METHOD

Patient recruitment: All patients who had been injected with BTX-A and were attending the Movement Disorder Clinic, Siriraj Hospital, Mahidol University Bangkok, Thailand from 1989 to 1994 were analysed for demographic data, indication for BTX, site of injection, amount of BTX given, response rate, duration of response and complications. The grand total of 900 patients from the data bank of the Movement Disorder Clinic were retrieved for analyses. The duration of follow-up of each patient ranged from 6 months to 6 years.

Botulinum A toxin was supplied initially by Smith-Kettlewell Eye Research Institute, San Francisco (1989-1992) and later by Allergan Inco-peration, California (1992-1994). Preparation of BTX-A was done half an hour prior to the injection and never used after 6 hours of preparation to ensure its efficacy. Every vial of BTX-A was kept in the deep freeze under minus 20° Celcius. The freeze-dried white powder toxin in each vial (contained 100 International Units) was diluted with 1 millilitre of saline solution as a preparation for injection i.e. concentration of 100 IU/ml. The amount, and site of BTX-A injection were adjusted according to the indication of each individual patient.

Statistical analyses in this study were expressed mainly as mean with standard deviation and percentage as appropriate.

RESULTS

Nine hundred patients from the Movement Disorder Clinic, Siriraj Hospital have been treated with BTX-A injection since 1989. The indications for BTX-A injection are tabulated in Table 1.

Table 1. Indications for BTX-A injection in 900 patients at Siriraj Hospital

Indications	Patients	Percentage
1. Hemifacial spasm	592	65.78
2. Occupational cramp	92	10.22
3. Blepharospasm and Meige syndrome	79	8.78
4. Spasmodic torticollis	72	8.00
5. Hemidystonia and generalised dystonia	19	2.11
6. Spasmodic dysphonia	11	1.22
7. Spastic hemiparesis	10	1.11
8. Miscellaneous i.e. tics, Gilles de la Tourette, facial myokimia, benign fasciculation, etc	25	2.78
Grand total	900	100

The amount of BTX injection for hemifacial spasm was 30 IU given at standard sites as adopted by Siriraj Hospital regime as in Fig. 1 whereas BTX injection for blepharospasm and Meige syndrome required 30-50 IU as in Fig. 2. Spasmodic torticollis required 30-120 IU of BTX-A injection into the most overactive group of muscles which were responsible for abnormal neck posture (mainly sternoclidomastoid and splenius capitis) as in Fig. 3 and 4. Most occupational cramp patients were writer's cramp with only one exception, a musician cramp. The total amount of BTX-A injection per patient for writer's cramp ranged from 40 to 80 IU, given in 2-4 divided doses over the overactive forearm muscles observed during writing without electromyographic glidance.

For spasmodic dysphonia patients, 2-3 IU of BTX-A injection was given directly into one side of the spastic vocal cord only *via* the percutaneens route through the cricothyroid membrane

Fig. 1 Th for at

Fig. 3 Injecti mastoi tortico

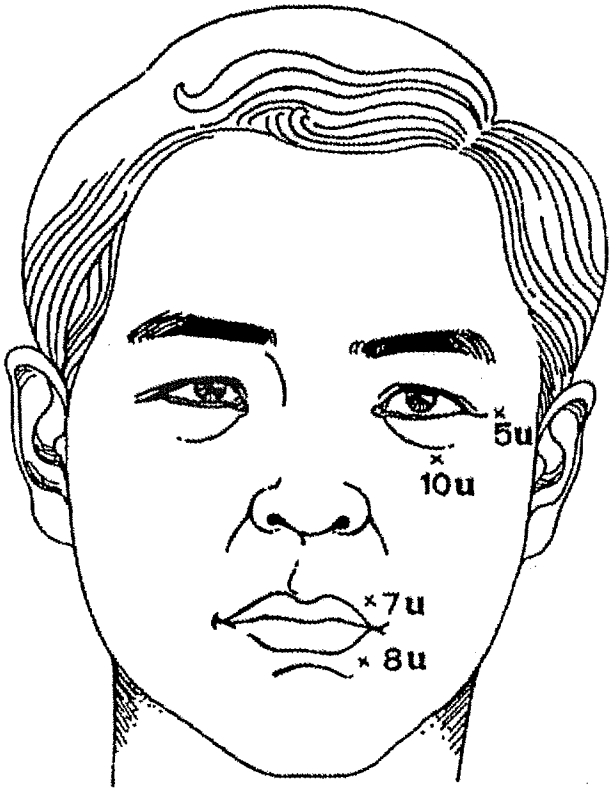

Fig. 1 The site and amount of BTX-A injection for patient with hemifacial spasm adopted at Siriraj Hospital.

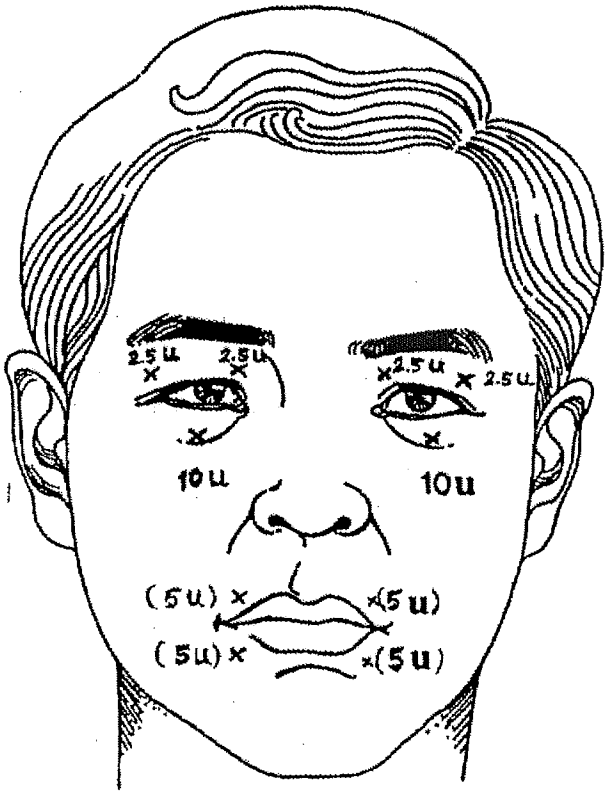

Fig. 2 The site and amount of BTX-A injection for patient with blepharospasm and Meige syndrome treated at Siriraj Hospital.

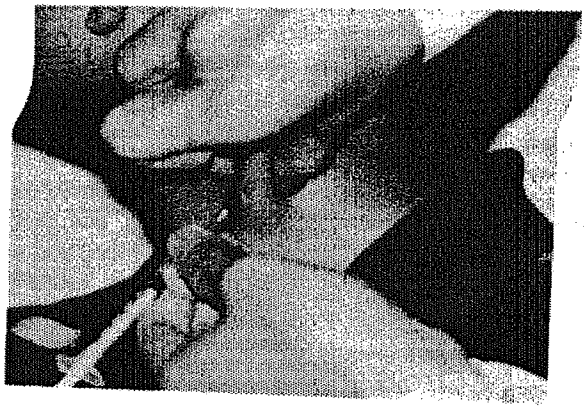

Fig. 3 Injection of BTX-A into the sternocleidomastoid muscle for patient with spasmodic torticollis.

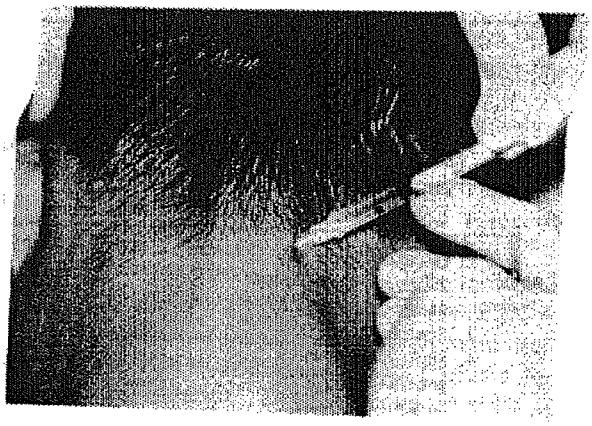

Fig. 4 Overcontracted posterior neck muscle was injection with BTX-A for patients with spasmodic torticollis.

by a well trained otolaryngologist. Patients with hemidystonia and generalised dystonia were also given BTX-A injection for symptomatic treatment over the most troublesome group of muscles with the total dosage ranging from 50 to 100 IU.

Recently 10 spastic hemiparesis patients due to previous cerebrovascular disease with severe disability and painful spasm were treated with BTX-A injection directly into adductor magnus, biceps, flexor digitorum profundus in the total amount of 100 IU per patient. Miscellaneous indications for BTX-A injection at Siriraj Hospital were tics, Gilles de la Tourette, facial myokimia, benign fasciculaton, lumbosacral strain with back muscle spasm and foot dystonia.

The results of BTX-A treatment in 592 hemifacial spasm patients are tabulated in Table 2.

Table 2. The results of BTX-A treatment in 592 hemifacial spasm patients

Outcome	Patients	Percentage
1. Excellent (more than 50% improvement)	486	82.09
2. Moderate improvement (25 to 50% improvement)	60	10.14
3. Mild improvement (less than 25% improvement)	39	6.59
4. No improvement or worse or loss of follow-up	7	1.18
Total	492	100

Complications of BTX-A injection for 592 hemifacial spasm patients were noted in 50 patients (8.45 per cent) as mild transient facial weakness and 12 patients (2.02 per cent) with mild ptosis, from which they recovered within a few weeks. The effect of BTX-A treatment for hemifacial spasm lasted for 3-6 months duration.

Two-thirds of occupational cramp and spasmodic torticollis patients who had BTX-A injection were reported as definite improvement whereas blepharospasm, Meige syndrome, spasmodic dysphonia, spastic hemiparesis, hemidystonia, generalised dystonia and tics responded in 79-88 per cent of the patients as shown in Table 3. Effectiveness of BTX-A injection lasted for 2-6 months depending on the amount of injection as well as type of clinical indications for injection.

DISCUSSION

Muscular spasms and abnormal involuntary movements are among the most troublesome human afflictions. One of the most disabling forms of muscular spasm is dystonia. Dystonia is a neurological syndrome manifested by involuntary sustained contractions or spasms of muscle producing abnormal movements and postures. Common types of dystonia include blepharospasm (spasms of eyelids causing involuntary eye closure), spasmodic torticollis (spasm of neck muscles causing twisting movements, abnormal posture and neck pain), spasmodic dysphonia (spasms of vocal cords causing strained voice), task-specific dystonias (writer's cramp), and oromandibular dystonia (spasm of mouth and jaw muscles causing grinding of teeth and secondary temporomandibular

Table 3. Response rate of BTX-A injection for various movement disorders

	Number	Loss of follow-up	Good response	Per cent
1. Occupational cramp	92	3	62	67.39
2. Blepharospasm and Meige syndrome	79	5	65	82.28
3. Spasmodic torticollis	72	4	49	68.06
4. Hemidystonia and generalised dystonia	19	3	15	78.95
5. Spasmodic dysphonia	11	0	9	81.82
6. Spastic hemiparesis	10	0	8	80.00
7. Miscellaneous i.e. tics, Gilles de la Tourette, facial myokimia, benign fasciculation, etc.	25	1	22	88.00

joint syndrome as well as speech, chewing and swallowing difficulties). Besides dystonia, there are many other causes of muscular spasms such as hemifacial spasm, tremor, tics, myoclonus, spasticity and various musculoskeletal spasms.

The approach of using botulinum toxin began in 1970-1971, by Allan B Scott for the treatment of strabismus and its use subsequently expanded to other conditions manifested by involuntary muscle spasms, including blepharospasm and other focal dystonias, hemifacial spasm, spasticity, and tremors. The use of BTX in the treatment of these and other conditions associated with undesirable muscle contractions represent one of the most important advances in neurological therapeutics.

The impact of this therapeutic intervention on functioning of patients with involuntary muscle spasms of various origins has been enormous. As a result of BTX therapy, patients with blepharospasm, for example, are now able to read, drive and watch television; patients with hemifacial spasm are no longer irritated and embarassed by involuntary facial twitches; patients with oromandilbular dystonia are able to chew and no longer experience grinding of their teeth and discomfort associated with temporomandibular joint syndrome; patients with cervical dystonia have less neck pain and are able to control their head position; patients with spasmodic dysphonia are able to speak in public and on the telephone without strain and strangulation; patients with writer's cramp and tremor can write and type; patients with tics no longer twitch and jerk; and patients with leg spasticity can ambulate better and have less discomfort from spasms.

In Thailand, we have adopted BTX-A injection for treatment of patients with various movement disorders since January 1989 at Siriraj Hospital under the approval of the Mahidol University Research Committee (before the American Food and Drug Administration approval of BTX in December 1989). Our series of 900 consecutive patients treated with BTX-A injection revealed hemifacial spasm as the most common condition (65.78 per cent), occupational cramps ranked second (10.22 per cent), blepharospasm and Meige syndrome ranked third (8.78 per cent), spasmodic torticollis ranked fourth (8.00 per cent) and miscellaneous conditions (2.78 per cent) ranked fifth respectively. The high prevalence of hemifacial spasm among the Asian population is striking but why it is so is still an unanswered observation. All Caucasian series of BTX-A injection revealed a higher number of patients with blepharospasm than hemifacial spasm but both conditions were also not as common as spasmodic torticollis[28].

Our series from Siriraj Hospital is the largest number of BTX-A treated patients for hemifacial spasm with the excellent outcome of 82.09 per cent. The complications of BTX-A injection for hemifacial spasm were noted in 8.45 per cent as mild transient facial weakness and 2.02 per cent as mild ptosis which recovered within a few weeks. The pattern and amount of BTX-A injection for hemifacial spasm is now established at Siriraj Hospital (Fig. 1) and it was adopted in other centres around the world after our previous report of 250 patients treated with BTX-A injection[29].

Occupational cramp and spasmodic torticollis are the two most difficult conditions to treat with BTX-A injection, because there are several clinical patterns as well as varying muscles affected. Most of the overcontracted muscles in occupational cramp are the deep seated group and need experience to identify the affected muscles for injection especially when this is done without electromyographic glidance[30,31]. Our previous report on BTX-A injection for 25 writer's cramp patients showed major functional improvement in 66.7 per cent which is similar to our recent experience and other series[30-36]. Our previous report of 56 spasmodic torticollis patients treated with BTX-A injection also showed marked improvement in 69.26 per cent[37].

Patients with blepharospasm and Meige syndrome responsed well to BTX-A injection (success rate of 82.28 per cent) and the effectiveness lasted for 2-4 months duration. Other series from the world literature revealed 77-100 per cent improvement of clinical symptoms and signs of blepharospasm after BTX-A treatment[38].

Patients with spasmodic dysphonia seemed to be the most cost-benefit effective for BTX-A injection because of the small amount of toxin used (only 2.5-5 IU) to correct laryngeal dystonia for 2-3 months duration. The technique of BTX-A injection adopted at our centre is *via* direct injection through the cricothyroid membrane into the thyroarytenoid muscle in one side only without electromyographic glidance. This pattern of injec-

tion was done by well trained otolaryngologists at our centre after practicing with several cadaver injections. The response rate of BTX-A injection for spasmodic dysphonia in our series was 81.82 per cent which was similar to other series[39].

Spastic hemiparesis patients from previous stroke also responsed well to BTX-A injection (80 per cent). Adductor magnus, biceps and deep flexor group of the forearm muscles were selected for injection according to the disability of each patient. The total amount of injection ranged from 60-100 IU.

Miscellaneous group of patients such as tics, Gilles de la Tourette, facial myokimia, benign fasciculation, back muscle spasm from sprain were also selected for BTX-A injection. The overal success rate of this miscellaneous group was 88 per cent.

Complications of BTX-A injection were transient, well accepted by the patient and no serious nor life threatening conditions occurred during the past 6 years of uninterrupted experience at our institute. Thus, it is now considered that BTX-A injection is a safe, and effective outpatient treatment for various types of movement disorders but it is still a costly therapy for Thai patients.

SUMMARY

A prospective open study of botulinum toxin A treatment for patients with various movement disorders at Siriraj Hospital, Mahidol University was analysed to evaluate its efficacy. The grand total of 900 patients comprised of a) 592 patients (65.78 per cent) with hemifacial spasm; b) 92 patients (10.22 per cent) with occupational cramp; c) 79 patients (8.78 per cent) with blepharospasm and Meige syndrome; d) 72 patients (8.00 per cent) with spasmodic torticollis; e) 19 patients (2.11 per cent) with hemidystonia and generalised dystonia; f) 11 patients (1.22 per cent) with spasmodic dysphonia; g) 10 patients (1.11 per cent) with spastic hemiparesis; and h) 25 patients (2.78 per cent) with miscellaneous group (i.e. tics, Gilles de la Tourette, facial myokimia, benign fasciculation, etc.).

The results of treatment for hemifacial spasm were classified as excellent in 486 patients (82.09 per cent), moderate improvement in 60 patients (10.14 per cent), mild improvement in 39 patients (6.59 per cent) and no improvement or worse in 7 patients (1.18 per cent). There were complications of mild transient facial weakness in 50 patients (8.45 per cent) and mild ptosis in 12 patients (2.02 per cent). The effect of botulinum toxin treatment lasted 3-6 months. In occupational cramp and spasmodic torticollis the good response rate was around two-thirds of all patients, whereas, blephalospasm, spasmodic dysphonia, spastic hemiparesis and tics responsed in 79-88 per cent of the patients.

Botulinum toxin A injection is thus a simple, safe, and effective out-patient treatment for patients with various kinds of movement disorders but it is a costly therapy.

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การรักษาผู้ป่วยที่มีการเคลื่อนไหวผิดปรกติด้วยการฉีดสารพิษชีวภาพโบทูลินัม เอ : ประสบการณ์ในการรักษาผู้ป่วย 900 ราย

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คณะผู้วิจัยได้ทำการศึกษาชนิดเปิด โดยการฉีดสารพิษชีวภาพโบทูลินัม เอ, รักษาผู้ป่วยกลุ่มที่มีการเคลื่อนไหวผิดปรกติในรูปแบบต่างๆกัน, ที่สาขาวิชาประสาทวิทยา, ภาควิชาอายุรศาสตร์, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล ระหว่างปี พ.ศ.2532-2537. วัตถุประสงค์ของการวิจัยเพื่อศึกษาประสิทธิผลของการรักษาวิธีดังกล่าวในผู้ป่วยทั้งสิ้น 900 ราย, ซึ่งประกอบด้วยผู้ป่วยที่มีภาวะใบหน้ากระตุกครึ่งซีก 592 ราย (ร้อยละ 65.78); ผู้ป่วยที่มีอาการหดเกร็งของกล้ามเนื้อเหตุอาชีพจำนวน 92 ราย (ร้อยละ 10.22); ผู้ป่วยตากระพริบเกร็งค้างและหรือมีกลุ่มอาการเม็ก 79 ราย (ร้อยละ 8.78); ผู้ป่วยคอบิดเอียงเกร็ง 72 ราย (ร้อยละ 8.00); ผู้ป่วยกล้ามเนื้อร่างกายหดเกร็งครึ่งซีกหรือหดเกร็งทั้งตัว 19 ราย (ร้อยละ 2.11); ผู้ป่วยพูดลำบากจากสายเสียงหดเกร็ง 11 ราย (ร้อยละ 1.22); ผู้ป่วยอัมพาตชนิดหดเกร็งครึ่งซีก จำนวน 10 ราย (ร้อยละ 1.11) และกลุ่มผู้ป่วยคละซึ่งประกอบด้วยผู้ป่วย tics, Gilles de la Tourette, facial myokimia, benign fasciculation เป็นต้น จำนวน 25 ราย (ร้อยละ 2.78).

ผลการรักษาพบว่าผู้ป่วยใบหน้ากระตุกครึ่งซีกมีการตอบสนองดีมากต่อการฉีดสารพิษชีวภาพ โบทูลินัม เอ 486 ราย (ร้อยละ 82.09); ดีปานกลาง 60 ราย (ร้อยละ 10.14), ดีเล็กน้อย 39 ราย (ร้อยละ 6.59) และไม่ได้ผลหรืออาการเลวลง 7 ราย (ร้อยละ 1.18). ผลแทรกซ้อนจากการรักษาในผู้ป่วยโรคใบหน้ากระตุกครึ่งซีกพบว่า, มีอาการปากเบี้ยวเล็กน้อยและเกิดขึ้นชั่วคราว 50 ราย (ร้อยละ 8.45) และมีหนังตาตกเล็กน้อย 12 ราย (ร้อยละ 2.02). ประสิทธิผลของการรักษาวิธีนี้สามารถมีระยะเวลาคงฤทธิ์ของยาได้นาน 3-6 เดือน. สำหรับกรณีผู้ป่วยที่มีอาการหดเกร็งของกล้ามเนื้อเหตุอาชีพและผู้ป่วยคอบิดเอียงเกร็งนั้น, มีการตอบสนองดีต่อการรักษาโดยวิธีนี้ โดยถือว่าได้ผลดีมาก ราวสองในสามของผู้ป่วยทั้งหมด. ผู้ป่วยตากระพริบเกร็งค้าง, ผู้ป่วยพูดลำบากจากสายเสียงหดเกร็ง, ผู้ป่วยอัมพาตชนิดหดเกร็งครึ่งซีกนั้น, มีการตอบสนองดีต่อการรักษาโดยวิธีนี้ โดยได้ผลราวร้อยละ 79-88 ของผู้ป่วยทั้งหมด.

ดังนั้นในปัจจุบันจึงสรุปว่าการฉีดสารพิษชีวภาพโบทูลินัม เอ ในการรักษาผู้ป่วยกลุ่มที่มีการเคลื่อนไหวผิดปรกติในรูปแบบต่างๆนั้น, เป็นวิธีที่ได้ผลดี, ง่ายและสะดวกในการรักษาแบบผู้ป่วยนอก, แต่วิธีดังกล่าวยังมีราคาค่ารักษาสูง.

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ELSEVIER

Anatomy of the Sweat Glands, Pharmacology of Botulinum Toxin, and Distinctive Syndromes Associated With Hyperhidrosis

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Abstract. *For a long period the therapeutic modalities to treat focal hyperhidrosis (HH) were very limited. Due to this the problem of focal HH was delt with stepmotherly. Nowadays we can consider BTX as the therapy of choice for axillary HH after topical treatment with aluminium salts have failed. The amount of successful reports on botulinum toxin (BTX) in the treatment of focal HH brought a change and the interest for this specific disorder grew. This article gives details on anatomy and physiology of sweating and mechanism of BTX. Further distinctive syndromes associated with HH, which all can be treated with BTX like localized unilateral hyperhidrosis (LUH), Ross' Syndrome and Frey' Syndrome are presented.*

A diagnosis of primary HH is usually based on the patients's history, typical younger age and visible signs of excessive sweating. Before treatment it is important to objectify focal HH with performing sweat tests such like Minor starch test and/or gravimetry. The total number of sweat glands is somewhere between 2 and 4 million and only about 5% are active at the same time, indicating the enormous potential for sweat production. The eccrine sweat gland is a long-branched tubular structure with highly coiled secretory portion and a straight ductular portion. Sweat is produced by clear and dark cells and is a clear hypotonic, odorless fluid. In response to nerve impulses, Acetylcholine (ACh) is released from the presynaptic nerve endings and then binds to postsynaptic cholinergic receptors presumably present in the basolateral membrane of the clear cells. This activates a complex in- and efflux of electrolytes creating the hypotonic sweat.

Injection of BTX leads to temporary chemodenervation with the loss or reduction of activity of the target organ. BTX is consisted of a heavy and a light chain. The structural architecture of BTX comprises three domains—L, H_N and H_C—each with a specific function in the mechanism of cell intoxication. The heavy chain is responsible for binding to the nerve cell, whereas the light chain catalyzes the proteolysis of one of the three SNARE proteins (Snap-25, Vamp or Syntaxin) depending to the serotype of BTX (7 serotypes A-G). Once cleaved by BTX, the SNARE proteins cannot become part of the complex capable of mediating the vesicle membrane fusion and therefore prevents the release of ACh and hence transmission of the nerve impulse.

Specifying an exact definition of hyperhidrosis is difficult, because there is no clear-cut distinction between physiological sweating and excessive pathological perspiration. The perception of hyperhidrosis is very individual. Some persons complaining of hyperhidrosis and demanding treatment demonstrate no objective basis for their perception of excessive sweat production. These persons may be suffering from a body dysmorphic disorder (ie, "botulinophilia").[1–3]

Hyperhidrosis may be defined as an excess of sweating beyond the amount required to return elevated body temperature to normal, with a distinction made between primary and secondary forms.[4] Primary (essential or idiopathic) hyperhidrosis involves focal hyperhidrosis, mainly of the palms, soles, axillae, and face. It is believed to be inherited and usually appears during adolescence. It arises mainly from emotional factors (ie, nervous sweating). In contrast, secondary hyperhidrosis is most often caused by underlying disease and induces general sweating. Common causes include chronic infection, neoplasia, and various endocrine diseases. Secondary hyperhidrosis may also be a sign of various neurologic diseases or a drug side effect.

A diagnosis of primary hyperhidrosis is usually based on the patient's history, typical younger age, and visible signs of excessive sweating. Sweat tests are useful in providing an objective parameter of hyperhidrosis and thus are indispensable before treatment. The extent of hyperhidrosis can be measured gravimetrically as the sweat rate, in milligrams per minute. The gravimetric test has been used exclusion criteria in the two important clinical studies on BTX treatment for

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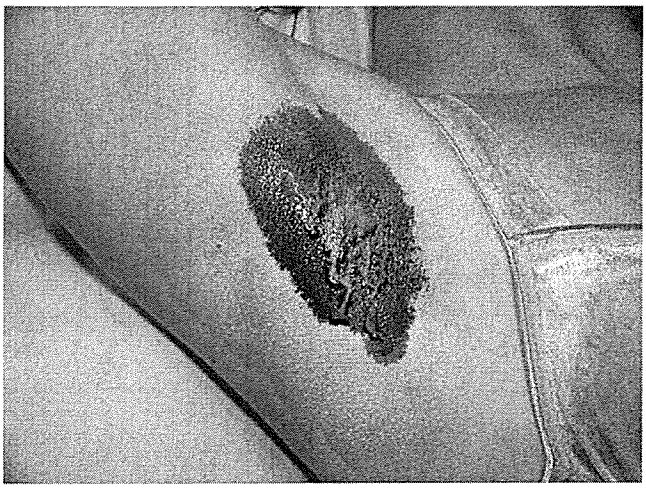

Figure 1. Sweat test after Minor. After application of iodine liquid followed by starch powder, the hyperhidrotic area becomes visible as a distinctive violet patch, enabling the clinician to precisely define the region of treatment as well as the intensity of hyperhidrosis.

axillary hyperhidrosis (exclusion <50 mg sweat per minute).[5,6] To establish the exact dimensions of the affected skin area the iodine starch test, according to Minor, should be performed in every case before treating patients with axillary hyperhidrosis with botulinum toxin (Fig 1).

Anatomy and Physiology of the Eccrine Sweat Glands

Anatomy

Eccrine sweat glands have an important thermoregulatory function, but they also respond to emotional stimuli. Under severe heat stress, they are capable of producing up to 10 L of sweat per day, but the normal secretion rate is 0.5–1 mL/min. The total number of sweat glands is somewhere between 2 and 4 million. Only about 5% of the sweat glands are active at the same time, indicating the enormous potential for sweat production.

The sweat glands are distributed over the nearly entire body surface except at the margins of the lips, nail beds, nipples, inner preputial surface, labia minora, glans penis, and glans clitoridis. They are most numerous on the soles (620 ± 20/cm^2), the forehead and the axilla (360 ± 60/cm^2), and the palms and the cheek (300 ± 80/cm^2). They are least numerous on the trunk (65 ± 20/cm^2) and the extremities (120 ± 30/cm^2).[7]

The eccrine sweat gland is a long-branched tubular structure with a highly coiled secretory portion and a straight ductular portion.[8] The duct fuses with the base of epidermal papillae to open via a rounded aperture onto the skin surface, which is visible as regular series of puncta along the centers of friction ridges.[7] The secretory coil, about 0.5 mm in diameter, is situated deep in the dermis or hypodermis, and both clear cells and dark cells can be distinguished within its epithelium. The clear cells secrete the major components of sweat, water and electrolytes. The dark cells are known to secrete glycoproteins, the most prominent protein constituents in sweat, but their other functions remain unknown.

Sweat is a clear, hypotonic, odorless fluid containing mainly sodium and chloride but also potassium, urea, lactate, amino acids, bicarbonate, and calcium. Proteins, such as immunoglobulins, constitute less than 1% of sweat by weight. The characteristic axillary malodor arises from bacterial decomposition of the sweat along with body danders and products of the apocrine sweat glands (lipids, cholesterol, and steroids).

The sweat glands are innervated by sympathetic nerve fibers. Nerve cells from spinal cord segments T2–T8 supply the skin of the upper limbs; those from T1–T4, the face and eyelids; those from T4–T12, the trunk; and those from T10–L2, the lower limbs.[7]

Physiology of Sweating

In response to nerve impulses, acetylcholine (ACh) is released from the presynaptic nerve endings and then binds to postsynaptic cholinergic receptors presumably present in the basolateral membrane of the eccrine gland clear cells. Activation of these receptors stimulates an influx of extracellular Ca^{++} into the cytoplasma. The increased intracellular [Ca] causes a KCl efflux from the cell. Consequently the cell shrinks, because water follows the solutes to maintain iso-osmolarity. The decrease in [K] and [Cl] leads to a transport of N^+, K^+, and 2 Cl^- into the cell in an electrically neutral fashion. The increase in cytoplasmatic [Na] stimulates the Na pumps to extrude cytoplasmatic Na^+ in exchange for extracellular K^+, so that in the steady state of secretion K^+ and Na^+ recyle across the basolateral membrane without further loss. In contrast, Cl^- enters the cell via Na-K-2Cl cotransporters and moves into the lumen through the Cl^- channels at the apical (luminal) membrane. This Cl^- movement depolarizes the apical membrane and generates the negative luminal potential, which attracts Na^+ into the lumen. Thus, Na^+ and Cl^- enter the lumen across the cell and join to form NaCl in the isotonic primary fluid (Fig 2). In the coiled portion of the sweat duct, reabsorption of NaCl occurs to preserve electrolytes, creating the hypotonic sweat, which is secreted to the skin surface.[9,10]

Physiology and Pharmacology of Therapeutic Botulinum Toxin

Injection of botulinum toxin (BTX) leads to a temporary chemodenervation with the loss or reduction of activity of the target organ. Of the seven known serotypes of

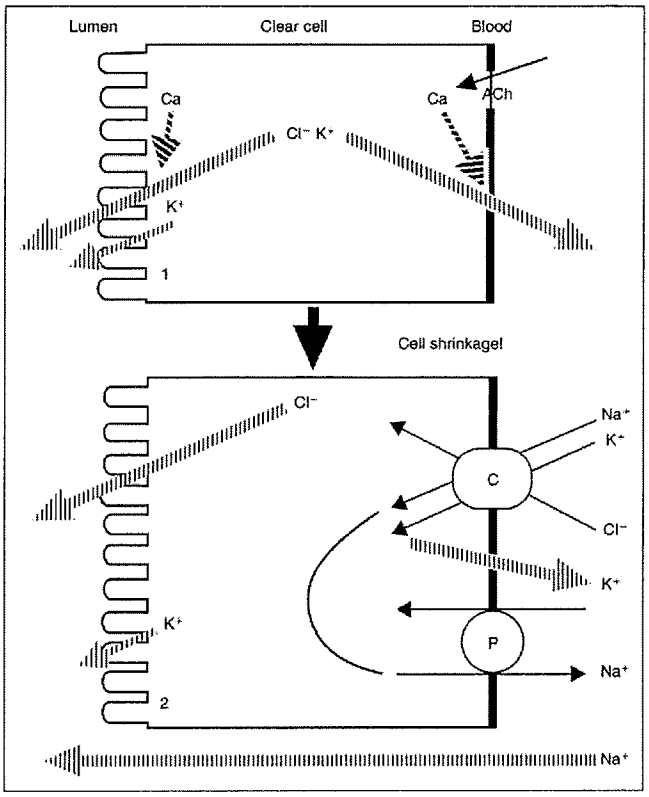

Figure 2. Modified scheme of the mechanism of eccrine sweat secretion from Hölzle.[10] C, Na-K-2Cl cotransporter; P, Na^+-K^+-ATPase–dependent Na pump.

BTX (A, B, C, D, E, F, and G), only serotypes A, B, E, and F are poisonous to humans. Types A and B are found in terrestrial environments; type E occurs in marine environments. Although the seven BTX serotypes are antigenically distinct, they have similar molecular weights and have a common subunit structure. BTX is synthesized as a single-chain polypeptide with a molecular mass of approximately 150 kDa. BTX is composed of a heavy chain (approximately 100 kDa) and a light chain (approximately 50 kDa) bonded by a disulfide bridge. Worldwide experience with BTX-A in form of Botox (Allergan Botox, Irvine, CA) or Dysport (Ipsen, Speywood Biopharm, Wrexham, UK) has demonstrated its safety and efficacy for numerous indications. Although both products are based on BTX-A, they have some important differences and thus should not be considered generic equivalents comparable by simple dose ratio. BTX-B, available as Neurobloc (Elan Pharmaceutical, Dublin, Ireland), may be useful in patients who do not respond to therapy with BTX-A.[11]

BTX neurotoxins are very specific for presynaptic nerve terminals because they bind only to receptors localized on the presynaptic membrane. BTX blocks the release of ACh from both skeletal and autonomic cholinergic nerve terminals. These bacterial metalloproteases act in the cell cytosol on selected proteins, but the

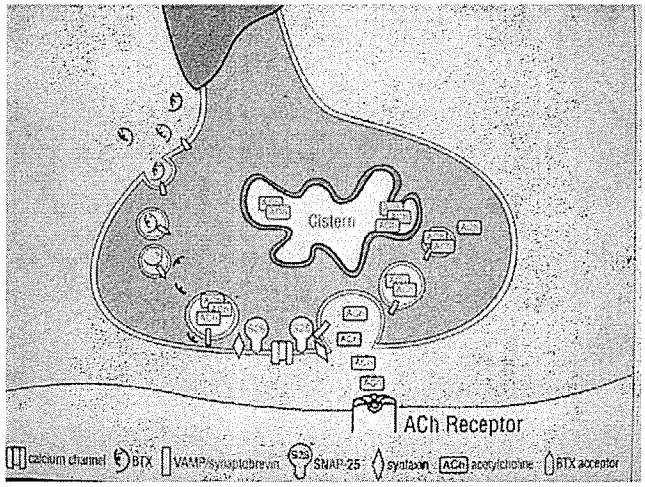

Figure 3. Entry and mechanism of action of BTX inside the presynaptic nerve ending. (Modified scheme Brin.[23]) Endocytosis of the intact BTX molecule to the presynaptic membrane is mediated by the H chain followed by the internalization inside endocytic vesicles. At low pH, BTX changes conformation, and the L chain is translocated into the cytosol, where it proteolyses one of the three SNARE proteins.

toxin must enter the nerve ending to exert its effect. Toxin-mediated paralysis involves three steps: (1) internalization, (2) translocation, and (3) inhibition of neurotransmitter (Ach) release (Fig 3).

The structural architecture of BTX comprises three domains—L, H_N, and H_C—each with a specific function in the mechanism of cell intoxication. The C-terminal half of the heavy chain determines cholinergic specifity and is responsible for binding, whereas the light chain provides intracellular toxic moiety. If the disulfide bond that links the two chains is broken before the cell can internalize the toxin, then the light chain cannot enter, and it loses its toxicity.

After binding with the heavy chain to the presynaptic membrane at as-yet unidentified receptors of peripheral nerve terminals, BTX is internalized inside endocytic vesicles. After internalization, the disulfide bond is cleaved by an unknown mechanism. Binding of the light chain to the vesicle membrane is mediated by the carboxyl-terminal domain (H_C),[12] whereas the N-terminal half of the heavy chain promotes penetration and translocation of the light chain across the endosomal membrane.

In response to activity of the ATPase proton pump, the endocytic vesicle becomes acid. At low pH, BTX changes conformation and inserts itself into the lipid layer of the vesicle membrane and translocates the light chain into the cytosol. Inside the cytosol, the L-chain catalyzes the proteolysis of one of the three SNARE proteins (Snap-25, Vamp, or Syntaxin) (Fig 3).[13]

The seven BTX serotypes are remarkably specific proteases. They cleave only one peptide out of thou-

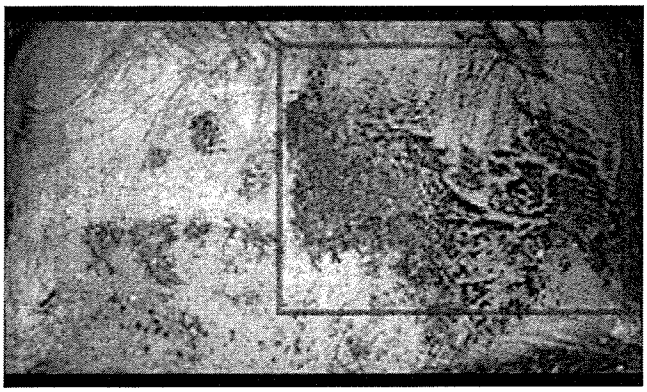

Figure 4. Restricted hyperhidrotic area on the forehead in a patient with localized unilateral hyperhidrosis.

sands of proteins present inside a nerve cell. BTX-A and -E cleave Snap-25; BTX-B, -D, -F, and -G cleave Vamp, and BTX-C cleaves both Syntaxin and Snap-25. Once cleaved by BTX, the SNARE proteins cannot become part of the complex capable of mediating the vesicle–target membrane fusion. At cholinergic nerve terminals, this specific proteolytic cleavage mediated by BTX in the synaptic cytosol prevents the release of ACh and hence transmission of the nerve impulse.[13]

Distinct Syndromes Associated With Hyperhidrosis

Besides primary and secondary hyperhidrosis, several rare forms of focal hyperhidrosis occur in association with specific syndromes. Some of these are of dermatologic interest, including localized unilateral hyperhidrosis (LUH), Ross' syndrome, and Frey's syndrome.

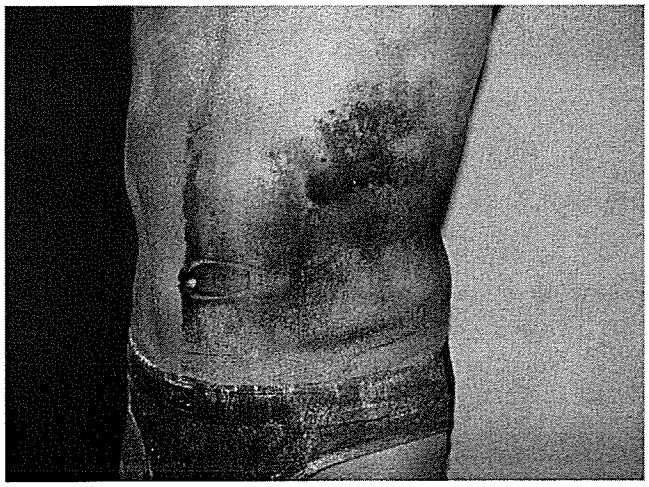

Figure 5. Ross' syndrome with compensatory sharply demarcated segmental hyperhidrosis due to an progressive segmental anhidrosis of the rest of the body.

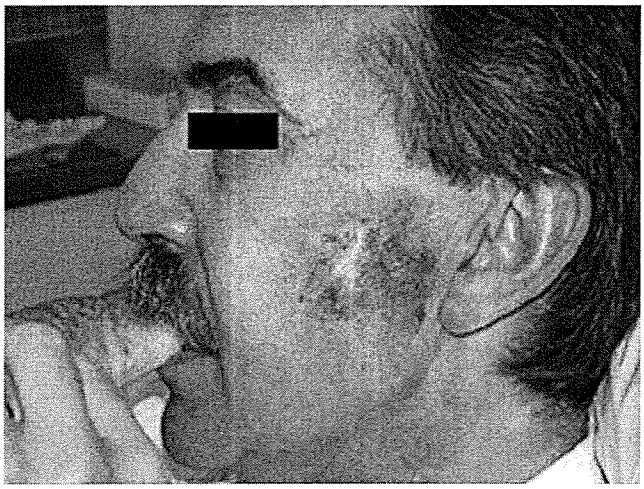

Figure 6. Gustatory sweating after parotid gland surgery. The intensity and extent of hyperhidrosis is demonstrated by iodine-starch test, after Minor.

Localized Unilateral Hyperhidrosis

LUH is a rare but well-defined form of localized hyperhidrosis (Fig 4) with an unknown pathogenesis that occurs in otherwise healthy people. It must be distinguished from unilateral segmental hyperhidrosis of the chest associated with thoracic malignancies[14] or from localized hyperhidrosis due to an eccrine nevus.[15] In LUH, hyperhidrosis usually occurs mainly on the forearm or the forehead and is restricted to an area smaller than 10 × 10 cm.[16] Beside the unusual localization, the major difference from primary hyperhidrosis is that LUH has no typical triggering factor and occurs even during sleep. The etiology of LUH is unknown; a misdirected reconnection of the sympathetic nerve fiber network after injury, similar to the pathogenesis of Frey's syndrome, has been postulated.[4] Therapy is not easy, but we reported a case of anhidrosis occurring after a 30-U Botox injection in a patient suffering from LUH.[4]

Ross' Syndrome

First described by the neurologist Alexander T. Ross in 1958,[17] this syndrome is characterized by the triad of unilateral tonic pupils, generalized areflexia (Holmes-Adie syndrome), and progressive segmental anhidrosis with a compensatory band of excessive perspiration. Patients with Ross' syndrome usually do not perceive the anhidrosis; instead, it is the compensatory segmental hyperhidrosis that is bothersome. In addition, many patients suffer from symptoms of vegetative dysfunction, including palpitations, stenocardia, orthostatic hypotonia, and irritable colon.[18] The pathogenesis of Ross' syndrome is unkown. Multiple neuropathies of the autonomous nervous system or a failure in the synthesis or release of neurotransmitters have been suggested as possible causes.[17] However, histologically there is no

evidence of nerve fiber destruction; therefore, Ross postulated a defect in ACh cholinesterase activity rather than in degeneration of sweat glands. The progression of Ross' syndrome is very slow, however. In 1992 Itin et al[19] reported a case of Ross' syndrome with a restricted area of anhidrosis to the right hand, right axilla, and right side of the face. In follow-up after 11 years, the anhidrotic areas progressed to the right hemithorax and the left lower extremity (Fig 5) (unpublished data). There is no therapy for the segmental progressive anhidrosis; however, the bothersome compensatory hyperhidrosis can be improved with systemic antimuscarinic drugs or with BTX injection into the main troublesome areas (ie, face).

Frey's Syndrome

Gustatory sweating is a common complication after parotid gland surgery, infection, or trauma. It was first described by Lucie Frey in 1923.[20] The most likely explanation for this condition is a misdirected resprouting of postsynaptic salivomotor parasympathetic fibers that have lost their glandular target organ. After gustatory stimulation, the clinical picture includes pathological sweating of preauricular area and also sometimes flushing as a reaction involving skin vessels (Fig 6). About 50% of patients experience gustatory sweating after parotidectomy, and about 15% of them consider their symptoms severe.[21] BTX is an effective therapeutic option for treating gustatory sweating and can be considered the first-line treatment. It is interesting to note that the duration of treatment effect in patients with Frey's syndrome (mean duration, 17.3 months) is much longer than in patients treated for other indications, such as hemifacial spasm or blepharospasm or even hyperhidrosis in other locations.[22]

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